LABORATORY

5

Cell Structure and Function

Learning Outcomes

5.1 Anatomy of a Human Cell

- Using a model or drawing, identify the parts of a human cell, and state a function for each part.

Prelab Question: Why is it beneficial for different parts of the cell to have specific functions?

5.2 Diffusion of Solutes

- Describe the process of diffusion as a physical phenomenon independent of the plasma membrane.
- Predict which solutes can cross the plasma membrane by diffusion and which cannot cross the plasma membrane by diffusion.

Prelab Question: Why is it beneficial for cellular substances to cross the plasma membrane by diffusion?

5.3 Osmosis: Diffusion of Water Across the Plasma Membrane

- Define osmosis, and explain the movement of water across a membrane.
- Define isotonic, hypertonic, and hypotonic solutions, and give examples in terms of NaCl concentrations.
- Predict the effect that solutions of different tonicities have on red blood cells.

Prelab Question: Why is 0.9% the usual tonicity of intravenous solutions?

5.4 Enzyme Activity

- Explain how enzymes have the ability to speed chemical reactions in cells.
- List some factors that can affect the speed of enzymatic reactions.

Prelab Question: Why is a warm body temperature advantageous to metabolism?

***Application for Daily Living:* Dehydration and Water Intoxication**

Introduction

Planning Ahead To save time, your instructor may have you start a boiling water bath and the potato strip experiment at the beginning of the laboratory.

We are accustomed to observing the outward appearance of human beings and rarely have an opportunity to become aware that humans are composed of microscopic entities called **cells.** Though we often think that the heart, liver, or intestines enable the human body to function, it is actually cells that enable these organs to work. In a previous lab, you observed human cheek cells (see Figure 3.8) using a compound light microscope. The much more powerful **electron microscope** led to the discovery that cells are actually very complicated because they contain many **organelles** that carry out enzymatic functions.

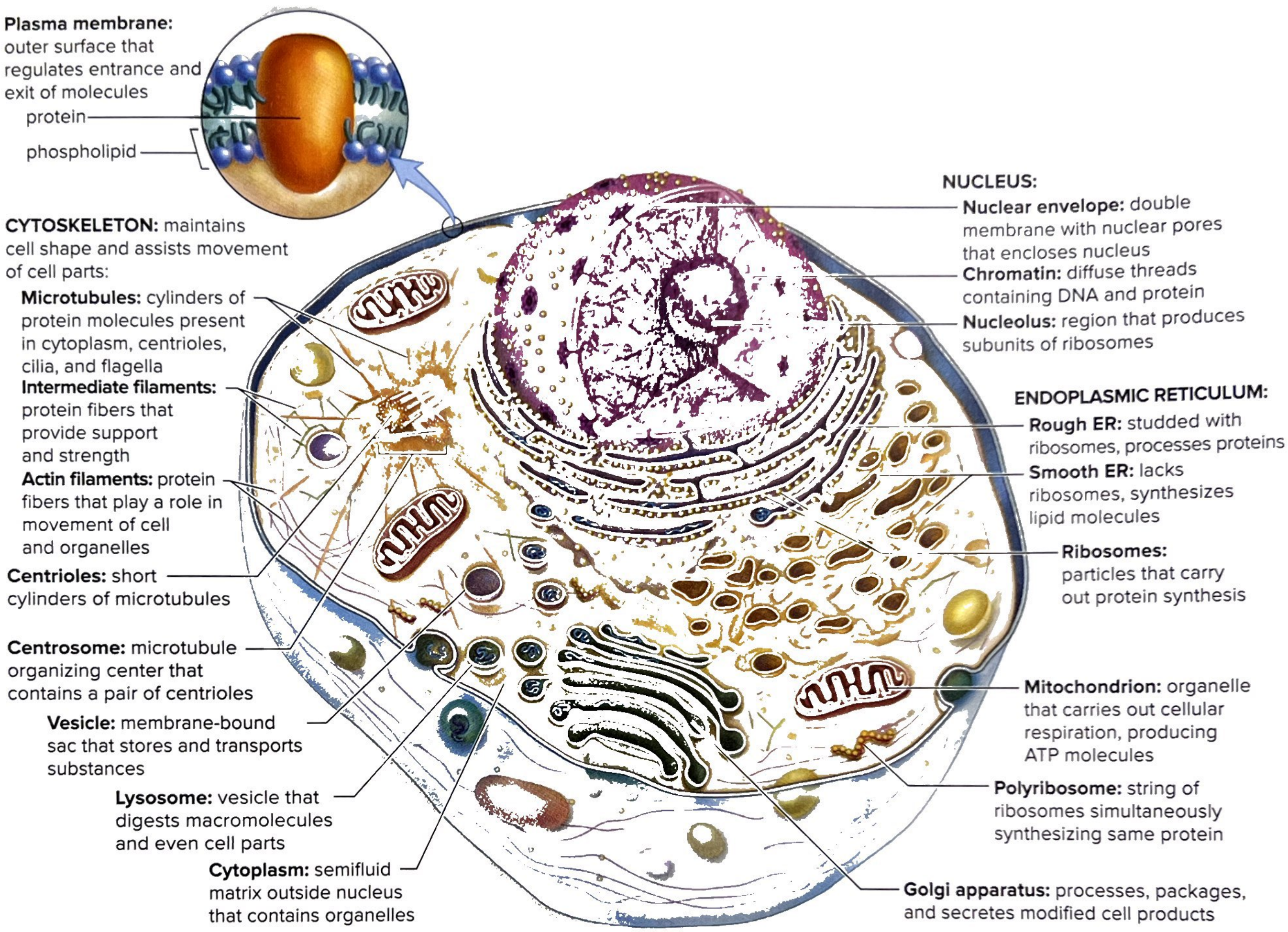
The model of an animal cell available in the laboratory is based on electron micrographs. In today's laboratory, we review the structure and function of **organelles,** subcompartments of a cell, before actually observing how its outer surface, the **plasma membrane,** serves as a selective regulator of what enters and exits cells. We will discover that the passage of water into a cell depends on the difference in concentration of solutes (particles) between the **cytoplasm** (contents of a cell) and the surrounding medium or solution.

Enzymes are proteins that carry out metabolic reactions within organelles, and we will have an opportunity to marvel at the speed of an enzymatic reaction, even at room temperature.

5.1 Anatomy of a Human Cell

Figure 5.1 shows that an animal cell is partitioned into a number of compartments. Just as a house works more efficiently when each room has a specialized function, so does a cell with different compartments for varied functions.

Figure 5.1 Human (animal) cell.



Learning the Organelles of the Cell

Identify these parts of a cell:

Composition and Function	Structure
1. Stack of membranous saccules; functions in processing, packaging, and distribution of molecules	______________
2. Membranous sacs; storage and transport of substances	______________
3. Have a double membrane; responsible for cellular respiration and production of ATP molecules	______________
4. Particles that carry out protein synthesis	______________
5. Outer surface that regulates entrance and exit of molecules	______________
6. Region in nucleus that produces subunits of ribosomes	______________
7. Short cylinders, present in centrosomes, of unknown function	______________
8. Central body, having diffuse threads of DNA and protein	______________
9. Vesicle that digests macromolecules and even cell parts	______________
10. Composed of microtubules, actin filaments, and intermediate filaments; responsible for the shape of the cell and movement of its parts	______________
11. Membranous saccules and canals having no ribosomes; synthesize lipid molecules	______________

Learning That the Organelles Work Together

1. Imagine that this cell produces digestive enzymes that are sent to the digestive tract:
   a. Which part of the endoplasmic reticulum would produce these enzymes? ______________
   b. How would they be transported to another part of the cell? ______________
   c. Which organelle would process and package these enzymes for export? ______________
2. Imagine that this cell produces a sex hormone (a lipid molecule):
   a. Which part of the endoplasmic reticulum would produce these lipid molecules? ______________
   b. How would they be transported to another part of the cell? ______________
   c. Which organelle would process and package this hormone for export? ______________
3. The nucleus produces the subunits of ribosomes.
   a. Where in the nucleus are the subunits produced? ______________
   b. What part of the nuclear envelope allows them to get out of the nucleus? ______________
   c. Where do the subunits go, and what happens to them? ______________

   ____

4. How a cell breaks down engulfed substances
   a. *Label Figure 5.1* where a vesicle is forming in order to take in a substance that will be digested.
   b. This vesicle will fuse with a ______________ that contains digestive enzymes.

5.2 Diffusion of Solutes

Diffusion is the random movement of molecules from the area of higher concentration to the area of lower concentration until they are equally distributed. A **solution** consists of a liquid solvent (most often water) and dissolved particles called solutes.

Environmental Factors and Diffusion of Molecules

If you spray a deodorant in one corner of the room, it will soon spread to fill the room because diffusion has occurred. (Notice, therefore, that diffusion can occur independently of a plasma membrane.) Environmental factors such as temperature and the composition of the medium can affect the speed of diffusion. Air offers the least resistance to the random motion of molecules, followed by a liquid, and then by any type of a solid.

Observation: Environmental Factors and Diffusion

You will calculate the speed of diffusion (1) through a semisolid gel and (2) through a liquid. Hypothesize whether you expect diffusion to occur faster through a semisolid or through a liquid, and give a reason for your hypothesis. ______________________________

____

Diffusion Through a Semisolid

1. Observe a petri dish containing 1.5% gelatin (or agar) to which a crystal of potassium permanganate ($KMnO_4$) was added in the center depression.
2. Obtain the length of time the crystal has been in the dish from your instructor. Write the time in minutes in Table 5.1.
3. Using a ruler placed over the petri dish, measure (in mm) the movement of color from the center of the depression outward in one direction. Record the distance in Table 5.1.
4. Calculate the speed of diffusion by dividing the number of millimeters by the number of minutes, and record the speed in Table 5.1.

Potassium permanganate, $KMnO_4$
$KMnO_4$ is highly poisonous and is a strong oxidizer. Avoid contact with skin and eyes, and wash combustible materials. If spillage occurs, wash all surfaces thoroughly. $KMnO_4$ will also stain clothing.

Diffusion Through a Liquid

1. Add water to a glass petri dish.
2. Place the petri dish over a thin, flat ruler.
3. With tweezers, add a crystal of potassium permanganate ($KMnO_4$) directly over a millimeter measurement line.
4. After 10 minutes, note the distance the color has moved in millimeters (Fig. 5.2). Record the length of time and distance moved in Table 5.1.
5. Calculate the speed of diffusion, and record in Table 5.1.

Figure 5.2 Process of diffusion.
Diffusion is apparent when dye molecules have equally dispersed.

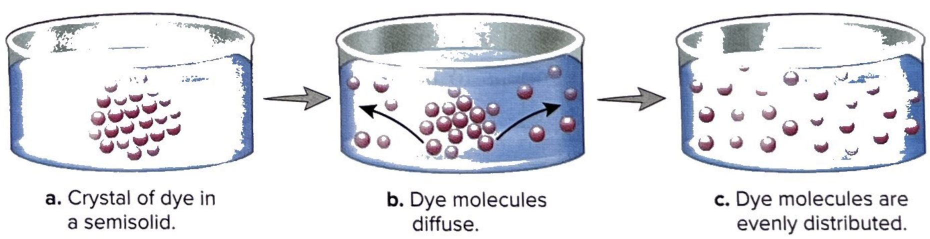


Table 5.1 Speed of Diffusion

Medium	Length of Time (min)	Distance Moved (mm)	Speed of Diffusion (mm/min)
Semisolid			
Liquid	10		

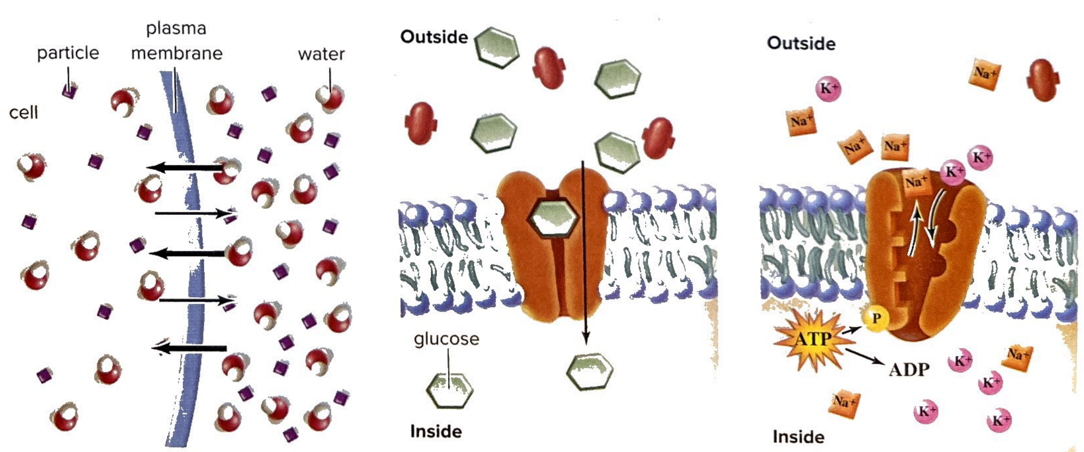
Conclusions: Diffusion

- Why did the dye molecules move rather than stay where they were originally? ______________________________
- In which experiment was diffusion the fastest? ______________________________
- What accounts for the difference in speed? ______________________________

The Plasma Membrane and Diffusion of Molecules

The plasma membrane regulates the passage of molecules into and out of cells. It is said to be **selectively permeable** because only small, noncharged molecules can diffuse across the plasma membrane without assistance (Fig. 5.3a). Carriers, proteins embedded in plasma membrane, can assist the passage of molecules across a membrane. Each carrier is specific to a particular molecule. During facilitated transport, a carrier (or a channel protein) assists a molecule diffusing across the membrane (Fig. 5.3b). Diffusion and facilitated diffusion require no energy because molecules move from areas of high concentration to areas of low concentration—that is, down a concentration gradient. Sometimes, substances need to be moved against the concentration gradient—from low to high. This occurs through active transport, where a carrier protein expends energy to move molecules across the plasma membrane.

Figure 5.3 Passage of molecules across a plasma membrane.
a. During diffusion, molecules move from the higher to the lower concentration. **b.** During facilitated transport, carrier proteins transport molecules from the higher to the lower concentration. **c.** During active transport, molecules move from the lower to the higher concentration; a protein carrier and energy are required.



a. Diffusion **b.** Facilitated transport **c.** Active transport

Experimental Procedure: The Plasma Membrane and Diffusion of Solutes

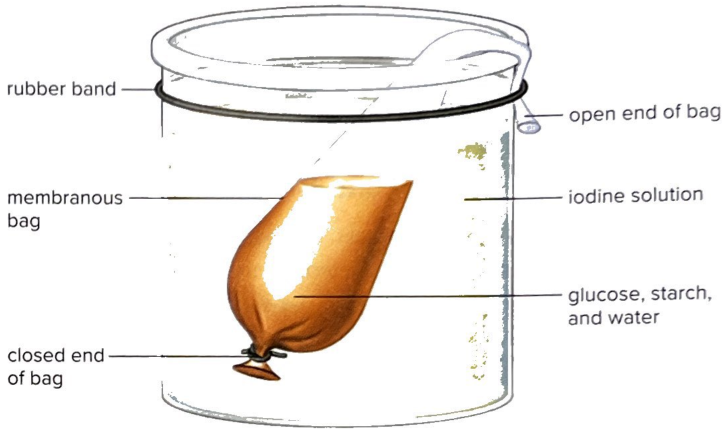
In this experiment, an artificial membrane is used to simulate the plasma membrane; the artificial membrane is also semipermeable. Only certain molecules can cross the membrane. The artificial membrane contains no carriers to assist the movement of molecules across the membrane.

Notice in Figure 5.4 that at the start of this experiment, glucose (small molecule) and starch (large molecule) will be inside the membranous bag and iodine (small molecule) will be outside the bag. Hypothesize which molecules will cross the membrane and in which direction they will move.

From outside to inside the bag ______________________________

From inside to outside the bag ______________________________

Figure 5.4 Diffusion experiment.



Diffusion Through a Selectively Permeable Membrane

At the start of this experiment,

1. Cut a piece of membranous tubing approximately 40 cm (approximately 16 in.) long. Soak the tubing in water until it is soft and pliable.
2. Close one end of the tubing with two knots.
3. Fill the bag halfway with glucose solution.
4. Add four full droppers of starch suspension to the bag.
5. Hold the open end while you mix the contents of the bag. Rinse off the outside of the bag with water.
6. Record the color of the bag contents in Table 5.2.
7. Fill a beaker 2/3 full with water.
8. Add droppers of iodine solution (IKI) to the water in the beaker until an amber (tealike) color is apparent.
9. Record the color of the solution in the beaker in Table 5.2.
10. Place the bag in the beaker with the open end hanging over the edge. Secure the open end of the bag to the beaker with a rubber band as shown (Fig. 5.4). Make sure the contents do not spill into the beaker.

After about 30 minutes, at the end of the experiment,

> **Benedict's reagent** Benedict's reagent is highly corrosive. Exercise care in using this chemical. If any should spill on your skin, wash the area with mild soap and water. Follow your instructor's directions for disposal of this chemical.

11. You will note a color change. Record the present color of the bag contents and the beaker contents in Table 5.2.
12. Obtain a small test tube. Using a graduated transfer pipette, draw 1 ml from the bottom of the beaker (near the bag), and place it in the test tube. Using a designated transfer pipette, add 3 ml of Benedict's reagent. Heat in a boiling water bath for 5 to 10 minutes, observe any color change, and record your results as positive or negative in Table 5.2. (Optional use of glucose test strip: Dip glucose test strip into beaker. Compare stick with chart provided by instructor.)
13. Remove the dialysis bag from the beaker. Dispose of it and the used Benedict's reagent solution in the manner directed by your instructor.

Table 5.2 Solute Diffusion Through a Membrane

	At Start of Experiment		At End of Experiment		
	Contents	**Color**	**Color**	**Benedict's Test (+) or (–)**	**Conclusion**
Bag	Glucose Starch			______	
Beaker	Water Iodine				

Conclusions: Plasma Membrane and Diffusion of Solutes

- Based on the color change noted in the bag, conclude what molecule diffused across the membrane from the beaker to inside the bag, and record your conclusion in Table 5.2.
- From the results of the Benedict's test on the beaker contents, conclude what molecule diffused across the membrane from the bag to the beaker, and record your conclusion in Table 5.2.
- Which molecule did not diffuse across the membrane from the bag to the beaker? ____________________

  Explain. ____

5.3 Osmosis: Diffusion of Water Across the Plasma Membrane

Osmosis is the diffusion of water molecules across a selectively permeable membrane. Like other molecules, water follows its concentration gradient and moves from a region of higher concentration to a region of lower concentration. To cross the plasma membrane, water passes through channel proteins called **aquaporins.** Therefore, osmosis (the diffusion of water) is a form of facilitated transport.

Tonicity is the relative concentration of solute (e.g., salt molecules) and solvent (water molecules) outside the cell compared to inside the cell.[1] An **isotonic solution** has the same concentration of solute (and therefore of water) as the cell. When cells are placed in an isotonic solution, there is no net movement of water. A **hypertonic solution** has a higher solute (therefore, lower water) concentration than the cell. When cells are placed in a hypertonic solution, water moves out of the cell into the solution. A **hypotonic solution** has a lower solute (therefore, higher water) concentration than the cell. When cells are placed in a hypotonic solution, water moves from the solution into the cell.

The next two Experimental Procedures explore tonicity using potato strips and red blood cells.

[1]Percent solutions are grams of solute per 100 ml of solvent. Therefore, a 10% solution is 10 g of sugar with water added to make up 100 ml of solution.

Experimental Procedure: Tonicity and Potato Strips

This procedure runs for 1 hour. Prior setup can maximize your time efficiency.

1. Cut two strips of potato, each about 7 cm long and 1½ cm wide.
2. Label two test tubes 1 and 2. Place one potato strip in each tube.
3. Fill tube 1 with water to cover the potato strip.
4. Fill tube 2 with 10% sodium chloride (NaCl) to cover the potato strip. NaCl is table salt.
5. After 1 hour, observe each strip for limpness (water loss) or stiffness (water gain). Which tube has the limp potato strip? ___________ Why did water diffuse out of the potato strip?

   ____

   Which tube has the stiff potato strip? ___________ Why did water diffuse into the potato strip?

   ____

   ____

Red Blood Cells (Animal Cells)

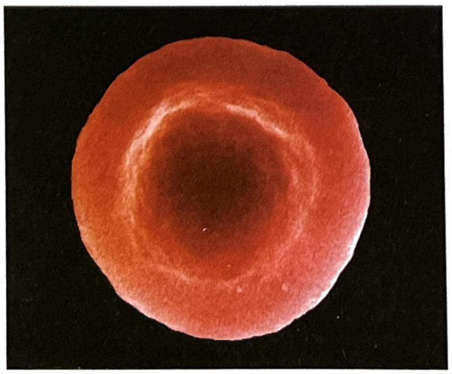
A solution of 0.9% NaCl is isotonic to red blood cells. In such a solution, red blood cells maintain their normal appearance (Fig. 5.5a). A solution greater than 0.9% NaCl is hypertonic to red blood cells. In such a solution, the cells shrivel up, a process called **crenation** (Fig. 5.5b). A solution of less than 0.9% NaCl is hypotonic to red blood cells. In such a solution, the cells swell to bursting, a process called **hemolysis** (Fig. 5.5c).

Complete Table 5.3 by following these instructions. In the second column, state whether the solution is isotonic, hypertonic, or hypotonic to red blood cells. In the third column, hypothesize the effect on the shape of the cell after being in this solution. In the fourth column, explain why you hypothesized this outcome. Base your explanation on the movement of water.

Table 5.3 Effect of Tonicity on Red Blood Cells

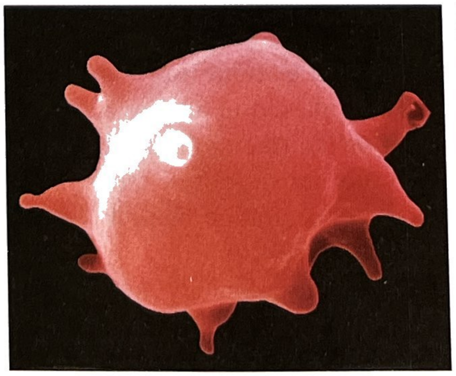
Concentration (NaCl)	Tonicity	Effect on Cells	Explanation
0.9%			
Higher than 0.9%			
Lower than 0.9%			

Figure 5.5 Tonicity and red blood cells.
(a–c) David M. Phillips/Science Source



15,000×

a. Isotonic solution. Red blood cell has normal appearance due to no net gain or loss of water.



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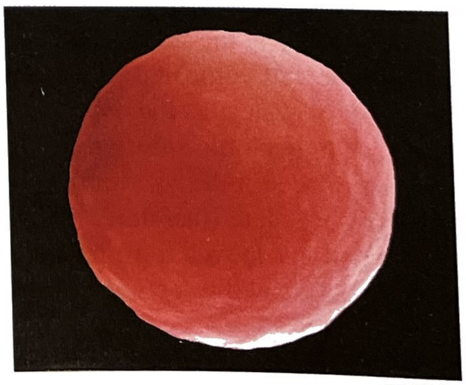
b. Hypertonic solution. Red blood cell shrivels due to loss of water.



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c. Hypotonic solution. Red blood cell fills to bursting due to gain of water.

Experimental Procedure: Tonicity and Red Blood Cells

Three numbered and stoppered test tubes are on display. Each test tube contains NaCl and a few drops of blood.

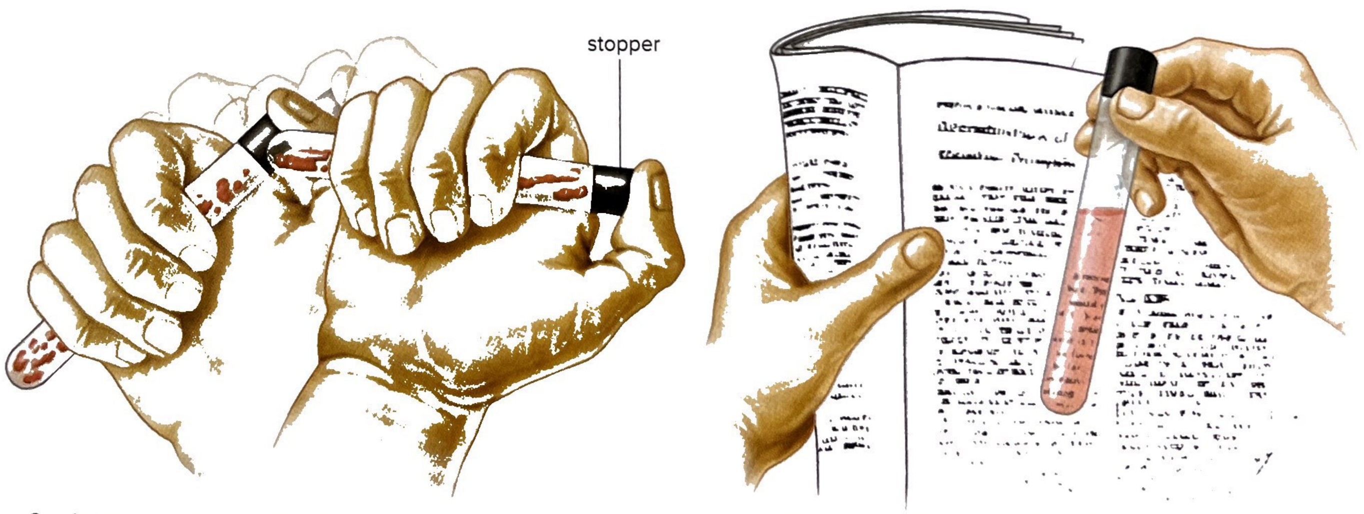
> ⚠ **Red blood cells** Do not remove the stoppers of test tubes during this procedure.

1. Shake each tube as shown in Figure 5.6a. Then place the tube in front of your lab manual as shown in Figure 5.6b. Determine whether you can see the print on the page, and record your decision under Print Visibility in Table 5.4.
2. Dependent on how difficult it is to see the print, which tube is hypotonic (less than 0.9% NaCl), hypertonic (10% NaCl), or isotonic (0.9% NaCl)? Record your deduction under Tonicity in Table 5.4.
3. In the last column of Table 5.4, explain how you arrived at this deduction.

Table 5.4 Print Visibility and Tonicity

Tube	Print Visibility	Tonicity	Explanation
1			
2			
3			

Figure 5.6 Proof of hemolysis.
a. Shake the tube as shown here. **b.** Once the red blood cells burst, you can read print placed behind a tube of diluted blood.



a. Gently invert tube several times.

b. Determine whether print is readable through tube.

5.4 Enzyme Activity

Enzymes are organic catalysts that speed metabolic reactions, either degradation or synthesis (Fig. 5.7). Each enzyme has a three-dimensional shape that accommodates its substrate(s), the reactant(s) in the enzyme's reaction. This shape, therefore, determines which substrate it will interact with and is important to the action of the enzyme. Although the shape of the enzyme and its substrate are compatible, the favored model for enzyme action suggests that the enzyme initially interacts with its substrate, changes shape slightly to improve the interaction, and then proceeds with a more efficient reaction. Certain environmental effects ensure that enzymes can function speedily. A warm temperature, sufficient enzyme and substrate concentrations, and the correct pH (whether the solution should be acidic, basic, or neutral) are all important. Each enzyme has a pH at which the speed of the reaction is optimum. Any pH higher or lower than the optimum affects the shape of the enzyme, leading to reduced activity.

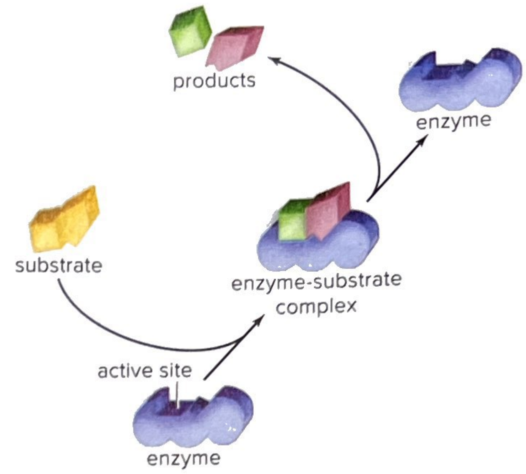


Figure 5.7 Enzymatic action. During degradation, the substrate is broken down into smaller products.

Experiment with the Enzyme Catalase

In the Experimental Procedure that follows, you will be working with the enzyme catalase. **Catalase** is present in cells, where it speeds the breakdown of the toxic chemical hydrogen peroxide (H_2O_2) to water and oxygen:

$$\underset{\text{hydrogen peroxide}}{2H_2O_2} \xrightarrow{\text{catalase}} \underset{\text{water}}{2H_2O} + \underset{\text{oxygen}}{O_2}$$

In this example, the enzyme is catalase; the substrate that fits within the active site of the enzyme is hydrogen peroxide; and the products are water and oxygen. Catalase performs a useful function in organisms because hydrogen peroxide is harmful to cells. Hydrogen peroxide is a powerful oxidizer that can attack and denature cellular molecules such as DNA. Knowing its harmful nature, humans use hydrogen peroxide as a commercial antiseptic to kill germs. In reduced concentration, hydrogen peroxide is a whitening agent used to bleach hair and teeth. It is also used industrially to clean almost anything from tubs to sewage.

Experimental Procedure: Catalase Activity

This Experimental Procedure tests the effects of pH on the activity of catalase. Potato will be the source of catalase. As the reaction occurs, easily observable bubbling will develop. Label two clean test tubes, and use the appropriate graduated transfer pipettes to follow these directions.

> **Protective eyewear** Protective eyewear should be worn for this procedure. HCl is a strong caustic acid. Exercise care in using this chemical, and follow your instructor's directions for disposal of this tube. If any acid should spill on your skin, rinse immediately with water.

Tube 1
1. Add 2 ml of distilled water to tube 1. (This is **neutral pH.**)
2. Smash a cube (1 cc) of potato using a mortar and pestle. Transfer to the tube.
3. Wait 3 minutes.
4. Add 4 ml of hydrogen peroxide. Record bubbling in Table 5.5 using 0 (no bubbling) or + signs (e.g., +, ++, +++, most bubbling).

Tube 2
1. Carefully add 2 ml of hydrochloric acid (5M HCl) to this tube.
2. Smash a cube (1 cc) of potato using a mortar and pestle. Transfer to the tube.
3. Wait 3 minutes.
4. Add 4 ml hydrogen peroxide. Record the degree of bubbling in Table 5.5.

Table 5.5 Effect of pH on Catalase Activity

Tube	Contents	Bubbling*	Explanation
1	Distilled water Smashed potato Hydrogen peroxide		
2	Hydrochloric acid Smashed potato Hydrogen peroxide		

*Ignore large bubbles and look for small bubbles.

Conclusions: Catalase Activity

- Give explanations for your results in Table 5.5.
- From your test results, decide whether enzymes can be negatively affected by environmental conditions, and explain your answer. ______________________________

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Application for Daily Living

Dehydration and Water Intoxication

Most people have heard of dehydration, but they may not realize that dehydration occurs because blood has become hypertonic to cells, and, in response, the cells lose water. Dehydration can be due to excessive sweating, perhaps during exercise, or it can be a side effect of many illnesses that cause prolonged vomiting or diarrhea. The signs of moderate dehydration are a dry mouth, sunken eyes, and skin that will not bounce back after light pinching. Most people have never heard of water intoxication, which occurs when blood becomes hypotonic to cells. In response to hypotonicity, cells gain water. Water intoxication can lead to pulmonary edema (the lungs gain water) and swelling in the brain. In extreme cases, it is fatal. It can be due to an intake of too much pure water during vigorous exercise, such as a marathon race. The cure is introducing an intravenous solution containing high amounts of sodium in a hospital setting. To prevent both dehydration and water intoxication, athletes should replace lost fluids slowly. Pure water is a good choice if the exercise period is short. Low-sodium solutions, such as sports drinks, are a good choice for longer-duration events such as marathons.

Laboratory Review 5

______________ 1. What is the function of rough endoplasmic reticulum?

______________ 2. Which organelle carries on intracellular digestion?

______________ 3. What is the function of the nucleus?

______________ 4. Which organelle is responsible for protein synthesis?

______________ 5. What regulates the movement of molecules into and out of the cell?

______________ 6. What term is used to describe the movement of molecules from an area of higher concentration to an area of lower concentration?

______________ 7. Which types of molecules can pass through the plasma membrane?

______________ 8. What is the term for the movement of water across a selectively permeable membrane?

______________ 9. Is 10% NaCl isotonic, hypertonic, or hypotonic to red blood cells?

______________ 10. What appearance will red blood cells have when they are placed in 0.0009% NaCl?

______________ 11. In which direction does water move when cells are placed in a hypertonic solution?

______________ 12. The active site of an enzyme brings together the ______________ of a reaction.

______________ 13. In general, what do unfavorable environmental conditions do to the speed of an enzymatic reaction?

______________ 14. An unfavorable pH causes an enzyme to lose its normal ______________.

Thought Questions

15. If a dialysis bag filled with water is placed in a starch solution, what do you predict will happen to the weight of the bag over time? Why?

16. Ocean water is hypertonic to the internal environment of the body. Predict what would happen to your cells if you drank large quantities of ocean (salt) water.

17. Why does the human body strive to maintain a near-neutral pH?